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Acrolein Induces Activation of the Epidermal Growth Factor Receptor of Human Keratinocytes for Cell Death

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Abstract Acrolein, which is a highly reactive formaldehyde generated by lipid peroxidation, can affect skin and cause various disorders. The effect of exposure of human keratinocytes to acrolein on cell surface-oriented signal transduction into cells was examined. Incubation of human keratinocytes with a relatively low concentration (50 μM) of acrolein caused a prompt and selective induction of tyrosine phosphorylation of the epidermal growth factor receptor (EGFR) as a 180-kDa molecule during the period from 5–30 min after the start of incubation. This early event was followed by an increase in the density and number of phosphotyrosine-containing proteins during the period from 60–120 min after the start of incubation. The catalytic activity of EGFR as measured by the levels of autophorphorylation and phosphorylation of an exogenously added substrate, casein, in in vitro kinase assay, greatly increased as early as 1 min after the start of incubation and then decreased gradually 30 min later. MAP family kinases, including ERK, JNK, and p38 kinase, and the potentially downstream transcription factor c-Jun were all promoted for phosphorylation/activation during a period of 5–30 min. Selective prompt phosphorylation/activation of EGFR followed by phosphorylation of MAP family kinases and c-Jun and their blockade by a specific EGFR inhibitor, AG1478, suggested that activation of EGFR is the major, and possibly single, cell surface element for intracellular signal transduction in acrolein-treated cells. Incubation of human keratinocytes with 50 μM of acrolein induced atypical apoptosis with morphologic apoptotic features with low-grade oligonucleoside-sized DNA fragmentation. Partial inhibition of such a cytopathic effect of acrolein on human keratinocytes by preincubation with AG1478 suggests the involvement of an EGFR-mediated signal pathway for atypical apoptosis. These results provide new information on acrolein-induced cell surface-oriented signal transduction to human keratinocytes, and this information may be useful for understanding the pathogenesis of a number of skin diseases in response to environmental acrolein and acrolein-generating ultraviolet irradiation. J. Cell. Biochem. 81:679–688, 2001.

Key words: acrolein; EGFR; human keratinocyte; cell death; signal transduction

Lipid peroxidation, which generates aldehydes highly reactive with various biomolecules including proteins, has been thought to contribute to the pathogenesis of various diseases such as atherosclerosis, diabetes, cancer, rheumatodis arthritis, postischemic-reoxygenation injury, and aging [Halliwell and Gutterridge, 1989]. Among the unsaturated aldehydes, acrolein shows the highest reactivity with the sulfhydryl group of cysteine, imidazole group of histidine, and amino group of lysine, resulting in the formation of stable acrolein-protein adducts [Esterbauer et al., 1991; Uchida et al., 1998]. Acrolein is generated as a proudct of organic pyrolysis and a metabolite of various chemical compounds and is thus a ubiquitus pollutant in the environment [Izard and Libermann, 1978]. In high organisms, acrolein is endogeneously proudced as a lipid peroxidation product [Alarcon, 1970; Timbrell, 1991].

Acrolein, which is present in tobacco smoke and automotive emissions, acts as a highly selective respiratory cartinogen [Grafstrom

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et al., 1994] and as a toxin that causes respiratory injury in humans, suppresses host defence against infections [ATSDR, 1990; WHO, 1992] and induces apoptosis in human alveolar macrophages [Li et al., 1997] possibly through downregulating NF-κB activity [Li et al., 1999]. Acrolein did not, however, induce typical apoptosis in lung carcinoma cell lines, although acrolein was highly cytotoxic to the cell lines [Rudra and Krokan, 1999]. Skin is another target of environmental acrolein, and is the site of endogenous production of acrolein from lipid following ultraviolet (UV) irradiation [Niyati-Shirkhodaee and Shibamoto, 1992]. In skin, acrolein may cause non-immunological contact hypersensitivity [Coverly et al., 1998; Verrier et al., 1999] and can display cytotoxicity and genotoxicity [Robinson et al., 1989; Dypbukt et al., 1993; Grafstrom et al., 1994]. Acrolein is known to act as a potent inhibitor of DNA excision repair enzymes, and this is believed to underlie the acrolein-mediated cytopathic effects [Robinson et al., 1989; Dypbukt et al., 1993; Grafstrom et al., 1994]. It is not known, however, whether acrolein triggers cell surface-oriented signal transduction potentially in linkage with dermatopathy. In this study, we demonstrated, for the first time, that acrolein affects human keratinocytes, causing activation of the epidermal growth factor (EGFR) and its downstream signal cascade, and that this EGFR-linked signal may partially mediate the acrolein-mediated cytopathic effect on keratinocytes.

MATERIALS AND METHODS

Chemicals

Acrolein (CH_2=CHCHO) was purchased from Aldrich Chemical Company Inc. (Milwaukee WI). AG1478, a specific EGFR inhibitor [Osherov and Levitzki, 1994; Levitzki and Gazit, 1995; Liu et al., 1999], was purchased from Calbiochem-Novabiochem Cor. (San Diego, CA).

Cell Cultures

Normal human keratinocytes were isolated from normal human skin tissues according to the method described by Matsumoto et al. [1997]. Briefly, human skin tissues obtained during plastic surgery with informed consent were treated with 1% Dispase (Godo Shusei Co., Ltd., Tokyo, Japan) in Dulbecco's modified Eagle's minimum essential medium (DMEM; Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) at 4°C overnight. The obtained human keratinocytes were cultured in Humedia-KG2 (Kurabo, Osaka, Japan) at 37°C. Second to fourth passage cells were used for our experiments. Cultures were maintained at 37°C in a humidified 5% CO_2 atmosphere.

For experiments to test the ability of acrolein to promote intracellular signal transduction, near-confluent cells were starved by incubation in growth supplement-free Humedia KG-2 for 12 h, washed once, and cultured in the same medium in the absence or presence of acrolein at 37°C.

Immunoblot, Immunohistochemistry, and Antibodies

Western blotting was performed according to the method described previously. The cell lysates (30 μg/lane) were subjected to sodium dodecyl sulfate-polyacrylamide gel (10%) electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes (Nihon Millipore Kogyo KK, Yonezawa, Japan). After the membranes had been reacted with the first antibodies, listed below, the reactions were examined by the Western Blot Chemiluminescence Reagent (DuPont NEN, Boston, MA). Anti-EGFR, anti-Jun N-terminal kinase (JNK), and anti-p38 mitogen-activated protein kinase (MAPK) polyclonal antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and anti-phosphotyrosine polyclonal, anti-extra cellular signal-regulated kinase (ERK) monoclonal antibody and anti-c-Jun monoclonal antibody were obtained from Transduction Laboratories (Lexington, KY). Expressions of phospho-ERK, phospho-JNK, phospho-p38, and phospho-c-Jun were examined using phosphoplus MAPK, phosphoplus JNK, phosphoplus p38, and phosphoplus c-Jun (Ser73) antibody kits (New England Biolabs, Inc., Beverly, MA), according to the manufacturer's instructions.

Immunoprecipitation and Kinase Assay

Immunoprecipitation was performed as described previously [Kato et al., 1998]. The immunoprecipitated EGFR proteins were washed three times with lysis buffer (30 mM Tris-HCl pH 8.0, 1% Triton-X-100, 150 mM NaCl, 1 mM EDTA, 0.5 mM Na_3VO_4) for either immunoblot or in vitro kinase assay. In vitro EGFR kinase assay was performed as described

previously [Kato et al., 2000]. Briefly, the immunoprecipitated EGFR proteins were washed three times with kinase buffer (10 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$), suspended in the kinase buffer with 1.5 mg casein (Sigma Chemical Co., St. Louis, MO) as an exogenous substrate, and radiolabeled with [γ-^{32}P] ATP (370 kBq) (NEN, Wilmington, DE). The kinase reaction was carried out for 20 min in a 30°C water bath and was terminated by adding sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerin) with (reducing) or without (non-reducing) 2-mercaptoethanol (2ME). The immunoprecipitates were then boiled for 3 min and loaded on 10% SDS polyacrylamide gels for electrophoresis. The gels were dried and exposed to Fuji X-ray film at −80°C for autoradiography. The molecular sizes of the developed proteins were estimated by comparison with protein molecular weight (MW) standards (Gibco, Gaitherberg, MD).

Detection of DNA Fragmentation

DNA fragmentation was detected as described previously [Herrman et al., 1994; Dohi et al., 1996]. In brief, cells were pelleted by centrifugation, and then 100 μM of lysis buffer containing 10 μM Tris-HCl (pH 7.4), 10 mM EDTA, and 0.5% Triton X-100 was added to the pellet and the preparation was left at 4°C for 15 min. After centrifugation at 15,000 rpm for 20 min at 4°C, the supernatant was incubated at 37°C with 0.8 mg/ml of RNase for 1 h followed by additional incubation for 1 h with 0.8 mg/ml of proteinase K. Then fragments of DNA were precipitated with 20 μl of 5 M NaCl and 120 μl of 2-propanol and left overnight at −20°C. Following centrifugation for 20 min, DNA fragmentation was analyzed by 1.4% agarose gel electrophoresis at 50 V for 1.5 h.

RESULTS

Acrolein Induces Tyrosine Phosphorylation of Multiple Proteins in Human Keratinocytes

First, we examined whether acrolein was capable of transducing an intracellular signal for inducing cellular protein tyrosine phosphorylation in human keratinocytes. The lysates of cells that had been incubated in the presence or absence of acrolein were subjected to SDS-PAGE and subsequent immunoblotting with anti-phoshotyrosine antibody. When 50 μM of acrolein was added to the culture of human keratinocytes and incubated for 5–120 min, the level of tyrosine phosphorylation of 180 kDa began to increase at 5 min after the start of incubation and continued to increase gradually up to 120 min after the start of incubation (Fig. 1A). The number and density of other phosphotyrosine-containing proteins were elevated only at 60–120 min after the start of incubation. A more striking increase in the density of multiple phosphotyrosine-containing proteins occurred in the keratinocytes incubated in the presence of 500 μM of acrolein (Fig. 1B), whereas 20 μM or lower concentrations of acrolein induced less-evident tyrosine phosphorylation of cellular proteins than did 50 μM (data not shown).

Acrolein Induces Tyrosine Phosphorylation and Activation of EGFR of Keratinocytes

The major protein band of 180 kDa that was rather selectively promoted for tyrosine phosphorylation within 30 min after the start of incubation in the presence of acrolein might correspond to EGFR, which could be the major receptor protein tyrosine kinase of keratinocytes. Therefore, we examined whether treatment of keratinocytes with acrolein induces tyrosine phosphorylation and activation of

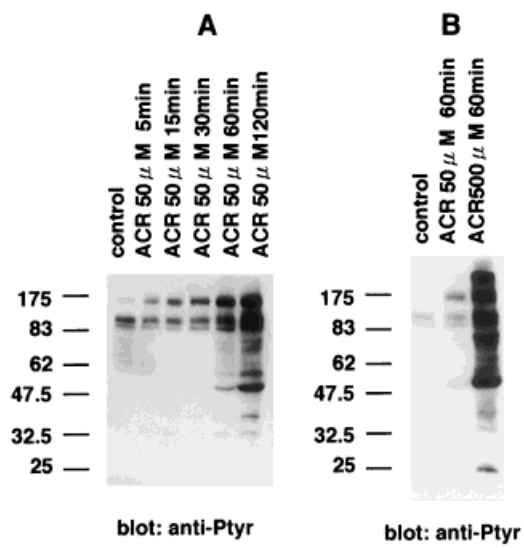

Fig. 1. Acrolein induces tyrosine phosphorylation of multiple proteins in human keratinocytes. Human keratinocytes were incubated with or without 50–500 μM of acrolein (ACR) for 5–120 min. The cells were lysed and subjected to immunoblot assay using an anti-phospho-tyrosine antibody. We performed three independent experiments with basically the same results, and only representative pictures are shown. The positions of moleculer mass marker proteins (kDa) are indicated on the left.

EGFR. We immunoprecipitated EGFR from the lysates of keratinocytes that had been incubated for 30 min with or without 50 μM of acrolein by use of specific anti-EFGR antibody and tested for the tyrosine phoshorylation level by immunoblotting with anti-phosphotyrosine antibody. The results are shown in Figure 2A. A clear increase in the tyrosine phosphorylation level by the acrolein treatment was observed with EGFR, which showed a 180-kDa band. We next performed in vitro kinase assay with the immunoprecipitated EGFR from the lysates of keratinocytes that had been incubated for 1–30 min with or without 50 μM of acrolein. The kinase activity of EGFR was measured by the levels of both autophosphorylation and phosphorylation of casein as an exogenous substrate. As shown in Figure 2B, a striking elevation in the in vitro kinase activity of EGFR developed as early as one minute after the start of incubation and then subsided up to 30 min after the start of incubation, although the autophosphorylation level was still higher than that of the control at 30 min after the start of incubation. These results demonstrated that acrolein

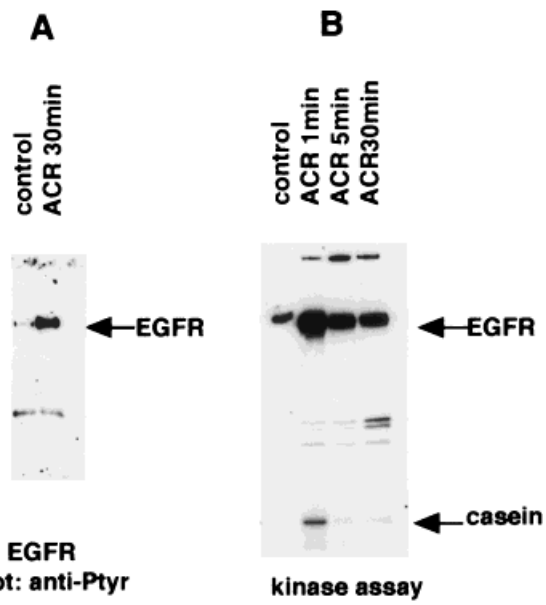

Fig. 2. Acrolein induces phosphorylation and activation of EGFR. Human keratinocytes were incubated with or without 50 μM of acrolein for the indicated times. Cell lysates were immunoprecipitated with anti-EGFR antibody, and immunocomplexes were subjected to immunoblot assay with anti-phosphoptyrosine antibody (**A**) or to in vitro kinase assay (**B**). We performed three independent experiments with basically the same results, and only representative data are shown. The positions of EGFR and the exogenous substrate casein (30 kDa) are indicated on the right.

is capable of inducing prompt activation of EGFR of keratinocytes.

Acrolein Activates MAP Family Kinases and c-Jun

EGFR, activated through binding EGF, is known to transduce an intracellular signal for activation of MAP family kinases, such as ERK, JNK, and p38 kinase, and a downstream transcription factor, c-Jun. Therefore, we tested whether acrolein-activated EGFR activates these signal transducing elements. The lysates of keratinocytes that had been incubated for 5–60 min with or without 50 μM of acrolein were subjected to SDS-PAGE followed by immunoblotting with phospho-MAPK/phospho-c-Jun-specific and MAPK/c-Jun-specific antibodies. The results are shown in Figure 3. The acrolein treatment of keratinocytes induced time-dependent increases in the levels of phospho-ERK, phospho-JNK, phospho-p38, and phospho-c-Jun but not in overall protein levels of ERK, JNK, p38, or c-Jun. The phospho-ERK level began to increase at 5 min after the start of incubation, peaked at 15 min, and then subsided upto 120 min after the start of incubation. In contrast, the level of phospho-JNK increased only slightly upto 15 min after the start of incubation, was markedly elevated at 30 min, and was further increased at 120 min. The pattern of increase in phospho-p38 was between those of ERK and JNK. The phospho-c-Jun level was elevated in parallel to the phospho-JNK level after acrolein treatment.

Evidence of Dependency on EGFR of the Acrolein-Mediated MAPK/c-Jun Activation

We next examined whether phosphorylation promotion/activation of cellular signal transducing elements is downstream of the EGFR activation. We used AG1478 as a known inhibitor of EGFR, which was previously confirmed to specifically inhibit the kinase activity of EGFR [Osherov and Levitzki, 1994; Levitzki and Gazit, 1995; Liu et al., 1999]. As shown in Figure 4A, the tyrosine phosphorylation of multiple cellular proteins induced by 60-min incubation with 50 μM acrolein was severely inhibited by preincubation of keratinocytes with 2 μM of AG1478 for 60 min. This result suggested that the majority, if not all, of protein tyrosine phosphorylation that had developed at 60 min after the start of incubation with acrolein depends on EGFR, which was quickly activated by the acrolein treatment. Corre-

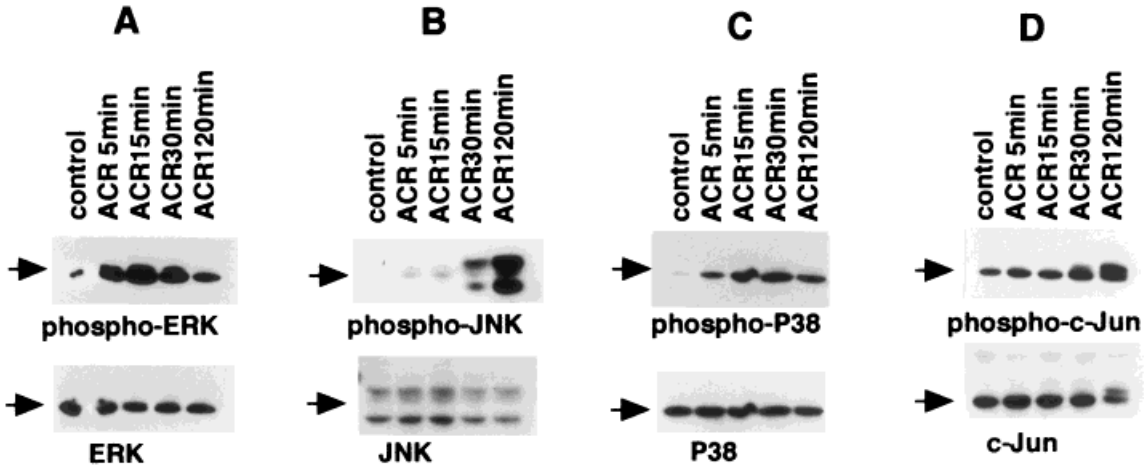

Fig. 3. Acrolein induces phosphorylation of MAP family kinases and c-Jun. Human keratinocytes were incubated with or without 50 μM of acrolein for the indicated times, and cell lysates were analyzed by immunoblot assay using the indicated antibodies. The membranes were first stained with anti-phospho-ERK, phospho-JNK, phospho-P38 or phosoho-c-Jun antibody (**top panels**). Then the membranes were stripped and restained with anti-ERK, JNK, P38 or c-Jun antibody (**bottom panels**). We performed three independent experiments with basically the same results, and only representative pictures are shown.

spondingly, pretreatment with AG1478 totally inhibited both background and acrolein-promoted phospho-ERK levels (Fig. 4B), suggesting a strong dependency of ERK phosphorylation on EGFR in keratinocytes. The same treatment also severely inhibited the acrolein-mediated promotion of phosphorylation of JNK and c-Jun, whereas it slightly less-extensively affected the acrolein-promoted phospho-p38 levels. These results suggested that the major pathway of acrolein-mediated signals for phosphorylation promotion and activation of signal transducing molecules starts at the EGFR activation.

Acrolein Induces Atypical Apoptosis of Human Keratinocytes Through a Partially EFGR-Linked Signal Pathway

The molecular mechanism of the known acrolein-associated cytotoxicity still remains to

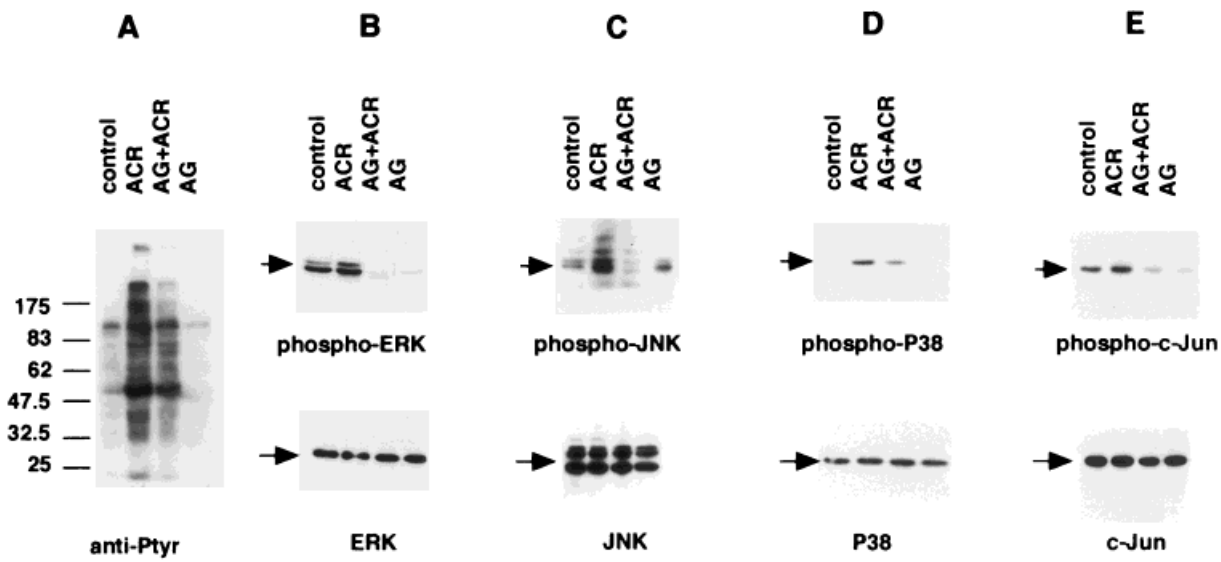

Fig. 4. Blockade of acrolein-induced protein phosphorylations by AG1478. Human keratinocytes were pre-incubated with 2 μM of AG1478 (AG) for 60 min. The AG-treated or untreated keratinocytes were further incubated with or without 50 μM of acrolein for 60 min, and cell lysates were analyzed by immunoblot assay using the indicated antibodies, as described in the legends of Figure 1 and Figure 3. We performed three independent experiments with basically the same results, and only representative data are shown.

be clarified. Acrolein has been reported to induce apoptosis in human alveolar macrophages [Li et al., 1997] but not in lung carcinoma cells [Rudra and Krokan, 1999]. We, therefore, tested whether acrolein induces apoptosis in human keratinocytes. DNA from keratinocytes that had been incubated in the presence of 50 μM acrolein for 24 h was subjected to analysis by agarose-gel electrophoresis. As shown in Figure 5, a low-grade DNA fragmentation developed in the acrolein-treated keratinocytes, in contrast to marked fragmentation with typical ladder formation in human T-leukemia cells (Jurkat cells) that had been similarly treated with acrolein. This result suggested that acrolein is capable of inducing DNA fragmentation in human T-leukemia cells but that keratinocytes are less sensitive to this action of acrolein than are T-leukemia cells. Nevertheless, incubation of human keratinocytes in the presence of 50 μM of acrolein, which induced activation of EGFR and downstream signaling elements, did induce morphologic changes in the keratinocytes for rounding-up and shrinkage in volumes of cells and cellular nuclei accompanied by development of apoptotic bodies (Fig. 6B, compared with Fig. 6A as a control), which corresponded to the morphologic features of apoptosis. It has been reported that, while DNA is degrated into fragments the size of oligonucleosomes in some cell types, with a ladder formation in agarose gel electrophoresis, larger DNA fragments are produced in others [Susin et al., 1999]. Taken together, the results suggest that acrolein induces atypical apoptosis in human keratinocytes. We next examined whether the acrolein-induced atypical apoptosis is linked to the signal transduction through EGFR. As shown in Figure 6C, the acrolein-induced morphologic changes were partially prevented by preincubation of the keratinocytes with AG1478. These results suggested that acrolein-mediated cytopathic action on keratinocytes in part involves EGFR-linked signal transduction.

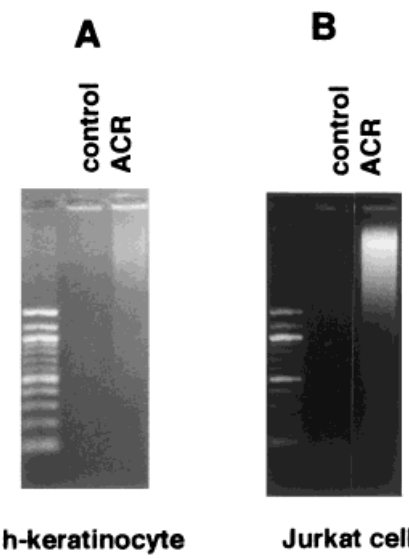

Fig. 5. Acrolein induces low-grade DNA fragmentation in humna keratinocytes. Human keratinocytes were incubated with or without 50 μM of acrolein for 24 h. For comparison, human T lymphoma cells (Jurkat cells) were also incubated with acrolein. Cells were lysed, and DNA samples were resolved in 1.4% agarose gel. We performed three independent experiments with basically the same results, and only representative data are shown.

DISCUSSION

We demonstrated that a relatively low concentration (50 μM) of acrolein clearly induced a rather long-lasing signal to increase the number and density of phosphotyrosine-containing proteins during a 120-min in vitro incubation of human keratinocytes, ultimately leading to atypical apoptotic cell death 24 h later. This concentration of acrolein was 1,000-times less than that of another carbonyl compound, glyoxal, needed for inducing comparable levels of protein tyrosine phosphorylation in murine thymocytes [Akhand et al., 1999]. A progressive increase in number and density of phosphotyrosine-containing proteins during 120 min of incubation with acrolein was also unique, compared with the results obtained for other chemical stimulants such as Hg^{2+}, which induced a quick response with a peak at 5 min [Rahman et al., 1993; Nakashima et al., 1994]. It was also noted that the acrolein-treated keratinocytes, EGFR, which formed a 180 kDa proten band in SDS-PAGE, was rather selectively affected by acrolein for tyrosine phosphorylation promotion during the initial 30 min of incubation and that only further promotion of EGFR phosphorylation during the period from 60–120 min after the start of incubation accompanied an increase in the number and density of other phosphotyrosine-containing proteins (Fig. 1). This suggested that EGFR was the major, and virtually the single, target of acrolein for tyrosine phosphorylation promotion

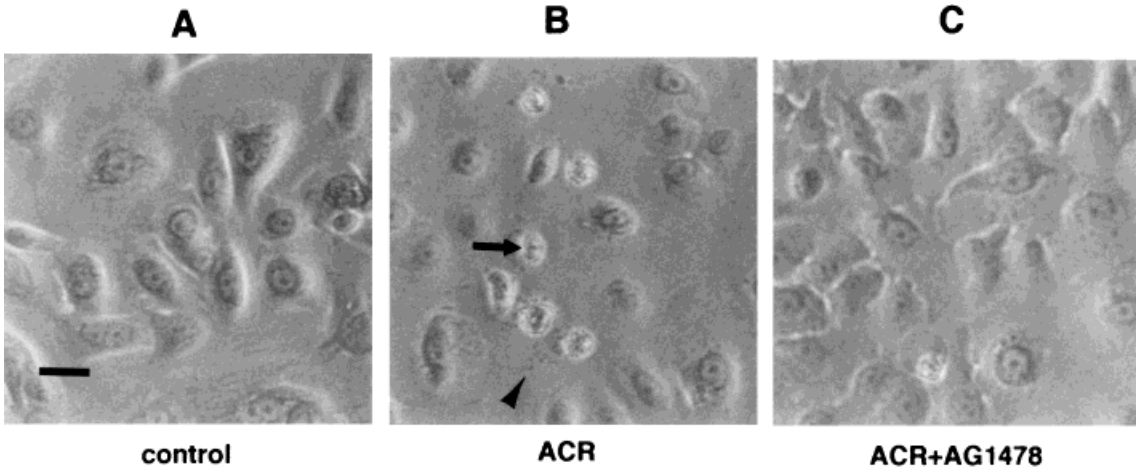

Fig. 6. Acrolein induces apoptotic morphological features in human keratinocytes in a partially AG1478-sensitive pathway. Human keratinocytes, some of which were preincubated with 2 μM of AG1478 for 60 min (panel **C**), were incubated with (panels **B** and C) or without (panel **A**) 50 μM of acrolein for 24 h. Morphology of these cells was observed under a phase-difference microscope. Note the rounding-up and shrinkage of a number of cells and nuclei in panel B (indicated by arrows), compared with their normal appearance in panel A. Apoptotic bodies (arrowheads) are seen here and there in the picture of panel B. These changes in cellular morphology after acrolein treatment were partially prevented by AG1478 as shown in panel C. We performed three independent experiments with basically the same results and took more than 50 photographs for each group, and only representative pictures are shown. Scale bar, 30 μm.

at the early stage of incubation. We then found that acrolein actually induces tyrosine phosphorylation and activation of EGFR as promptly as one minute after the start of incubation of keratinocytes with acrolein. The quickly elevated catalytic activity of EGFR after acrolein treatment, however, had subsided to a lower level 30 min later. This observation is in contrast to another finding that the tyrosine phosphorylation of EGFR gradually increased upto the end of 120-min incubation. The levels of autophosphorylation of EGFR and phosphorylation of other proteins, demonstrated by immunoblotting, probably reflect the summation of the phosphorylation during the whole incubation period, time possibly in the absence of powerful phosphatase activity. It has been reported that acrolein reduces glutathione contents in lung cells [Meacher and Menzel, 1999; Rudra and Krokan, 1999], making the cellular microenvironment oxidative. The oxidative microenvironment may downregulate the activity of protein tyrosine phosphatases through oxidization of the cysteine SH group in the active site of the catalytic domain [George and Parker, 1990; Stone and Dixon, 1994]. Therefore, the increase in the number and density of phosphotyrosine-containing proteins at a late stage after acrolein treatment might have resulted from both elevated kinase activity at an early stage and inactivation of protein tyrosine phosphatases at a later stage in the oxidative microenvironment.

We next showed that activation of EGFR by acrolein accompanied time-dependent phosphorylation/activation of three classes of MAP family kinases, ERK, JNK, and p38 kinase, as well as the potentially downstream transcription factor c-Jun. Again the concentration (50 μM) needed for activation of these intracellular signal-transducing elements was ten times less than that of another lipid-originated carbonyl compound, HNE [Liu et al., 1999]. The activation of ERK at an early time (starting at 5 min and peaking at 15 min) after the start of incubation with acrolein seemed to be independent of the time course of the increase in overall phosphotyrosine-containing proteins, which was evident only at 30 min after the start of incubation. However, it corresponded well to the quick activation of EGFR as a potential upstream event. The times of full activation of JNK, p38, and c-Jun were somewhat delayed compared with that of ERK and occurred at 30 min or later after the start of incubation. These observed time courses of activation of intracellular signal elements in keratinocytes, initiated by stimulation of 50 μM of acrolein, support the view that activation of EGFR is the major and central event among various acrolein-mediated

signaling events. This view was confirmed by the result that the majority, if not all, of the acrolein-mediated signaling events were inhibited by a specific EGFR inhibitor, AG1478.

The molecular mechanism of the activation of EGFR by a relatively low concentration of acrolein remains unknown. Earlier experiments showed that a number of chemicals that cross-link or make a cluster of cell surface molecules/receptors, such as Hg^{2+} [Nakashima et al., 1994], $NaAsO_2$ [Hossain et al., 2000], glyoxal [Akhand et al., 1999] and HNE [Liu et al., 1999], and reduction of cellular glutathione levels [Hossain et al., 2000; Liu et al., 2000] caused activation of both receptor and non-receptor protein tyrosine kinases, including Lck, Src and EGFR. Acrolein was not capable of inducing evident cross-linkage or clustering of EGFR (Takeuchi, unpublished communications). However, as stated above, acrolein can consume glutathione in the cell [Meacher and Menzel, 1999; Rudra and Krokan, 1999] and cause the intracellular microenvironment to become oxidative. The oxidative modification of the intracellular domain of tyrosine kinases has recently been shown to activate both receptor and non-receptor tyrosine kinases [Akhand et al., 1999; Kato et al., 2000]. It could therefore be that acrolein-mediated glutathione consumption caused oxidative modification of EGFR for activation.

EGFR, when stimulated by the natural ligand EGF, is known to transduce a mitogenic signal to most epithelial tissues and other cells [Williams et al., 1993; Fanger et al., 1997] as a growth factor-accepting receptor. However, a number of recent studies have suggested that the EGFR-mediated signal sometimes inhibits growth of these cells [Kawamoto et al., 1983; Fan et al., 1995; Liu et al., 1999]. As acrolein is known to be highly cytotoxic and EGFR has been shown to be the major target of acrolein in the present study, we hypothesized that acrolein-associated cytotoxicity might involve the EGFR-mediated signal transduction. We found that 50 μM of acrolein does display a cytopathic effect on keratinocytes for inducing morphologic features of apoptosis such as rounding-up and shrinkage of cells and nuclei with the development of apoptotic bodies accompanying low-grade DNA fragmentation into oligonucleosomes, and that this cytopathic effect was partially prevented by preincubation of the cells with a specific EGFR inhibitor, AG1478. Thus we conclude that the signal cascade initiated by acrolein-activated EGFR leads to atypical apoptotic cell death as a potentially alternative signal pathway for cell death induction to the previoulsy reported NF-κB-linked one [Li et al., 1999]. The presence of cascade to induce apoptosis not-accompanying typical DNA ladder formation has recently reported elsewhere [Susin et al., 1999]. The detailed signal cascade for atypical apoptotic cell death partially through EGFR in our system, however, remains to be clarified.

The results obtained in the present study may shed some light on the complex pathogenesis of skin, which frequently receives UV irradiation. UV irradiation to skin lipid has been shown to generate acrolein [Niyati-Shirkhodaee and Shibamoto, 1992] and to cause a number of pathologic skin conditions such as inflammation, cancer development, and aging [Robinson et al., 1989; Dypbukt et al., 1993; Grafstrom et al., 1994; Coverly et al., 1998; Verrier et al., 1999]. Till present we do not have any data that exactly measured the concentration of acrolein in the UV-irradiated local skin tissue. UV irradiation to local skin probably induces generation of acrolein whose concentration is lower than that used in the present study in vitro but is maintained for longer time through continuous production. Since a large portion of acrolein added into the culture of keratinocytes can be rapidly decayed to be inactive, the relations between concentration and effect in vivo and in vitro are difficult to be evaluated appropriately. Leaving this point for future study, the results of our present study suggest acrolein-activated EGFR plays an important role in some steps of the progression of one or more of these skin diseases.

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